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Genetic identification and color descriptions of early life-history stages of Belizean *Phaeoptyx* and *Astrapogon* (Teleostei: Apogonidae) with Comments on identification of adult *Phaeoptyx*

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Abstract

Phaeoptyx and *Astrapogon* are represented in the Caribbean by six species (*P. conklini, P. pigmentaria, P. xenus, A. alutus, A. stellatus,* and *A. puncticulatus*). Species identification of larvae and juveniles is problematic because characters used to distinguish adults (e.g., patterns of pigmentation and numbers of gill rakers) are absent, incomplete, or difficult to discern in the young stages. Neighbor-joining trees derived from mitochondrial cytochrome oxidase 1 sequences (DNA Barcoding) were used to match early life stages and adults. Subsequent comparative analysis of preserved voucher specimens from which the DNA was extracted or digital color photographs of those specimens taken prior to preservation yielded sufficient information to separate all early life-history stages of Belizean *Phaeoptyx* and *Astrapogon* and provided additional information for field identification of adult *Phaeoptyx*. Patterns of chromatophores in fresh material, combined with patterns of melanophores, provide the easiest means of separating the life-history stages of *Phaeoptyx*. Larvae of *Astrapogon* species are morphologically very similar, and some differences in pigmentation detected among them may reflect different stages of development. Continued implementation of the DNA Barcoding methods and field protocol outlined herein should prove valuable in accurately identifying much more of the ichthyoplankton fauna of the Caribbean.

Key words: DNA Barcoding, fish larvae, chromatophores, Belize

Introduction

To provide specific identifications of larvae of Caribbean reef fishes, we have been conducting field work for a number of years at the Smithsonian's research station at Carrie Bow Cay, Belize, a small coral-fringed island on the Belizean Barrier Reef ($16^0$48.5'N, $88^0$05'W). In recent years, we have augmented our protocol of rearing net-collected larvae through transformation (see Smith & Thacker 2000; Baldwin & Smith 2003) with matching larvae to adults through DNA Barcoding (Mitochondrial Cytochrome Oxidase 1 sequences). Among the identifications we have made genetically are early life stages of all species of the apogonid genera *Phaeoptyx* and *Astrapogon*, taxa that had previously presented identification problems. For *Phaeoptyx*, we had identified many more larval and juvenile morphotypes based on pigmentation than known species, and we did not know which features were significant for species identification. After matching the young stages to adults through DNA Barcoding, we were then able to go back to the voucher specimens and photographs of them and determine diagnostic characters for the young stages of all species.

The publication of *Early Stages of Atlantic Fishes* (Richards 2006) marked the most comprehensive effort to date to provide information for the identification of early stages of Western Central Atlantic fishes. The enormity of the subject matter, however, precluded detailed diagnoses and substantive comparative sections, useful information particularly for species for which early life stages have not already been described. Several

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original illustrations and brief descriptions of young stages of all species of *Phaeoptyx* and *Astrapogon*, except *A. alutus,* are included in this compilation (Lara 2006:1388–1399), but the information provided is insufficient for separating and identifying most of our morphotypes of young Belizean *Phaeoptyx* and *Astrapogon*. This is in part because color patterns of fresh material, which, in combination with melanophores, provide the easiest means of distinguishing most early life stages of apogonids, were not described.

In this paper, we describe our protocol for molecular identification of early life stages of Belizean fishes. Additionally, we provide the following: color photographs of larval, juvenile, and adult *Phaeoptyx* and larval *Astrapogon*; the first record of larval *A. alutus;* diagnostic features of all life-history stages of the three species of *Phaeoptyx*; diagnostic features of early life stages of *Astrapogon puncticulatus* and distinctive (but not necessarily diagnostic) features of larval *A. alutus* and *A. stellatus*; and comparative notes to help distinguish larval *Astrapogon* species and all life history stages of *Phaeoptyx*.

Methods

Juveniles and adults were collected in the Carrie Bow vicinity using the fish anesthetic quinaldine sulfate and dip nets. Larvae were collected in a plankton net of 505µm mesh fitted onto a 0.5 x 1 m rectangular frame made of pvc pipe and deployed from a dock at Carrie Bow. Juveniles were also obtained through rearing net-collected larvae in a flow-through seawater system. Adults were identified to species, and juveniles and larvae were identified to the lowest taxonomic level possible and sorted into types based primarily on chromatophore and melanophore patterns. Selected specimens were measured to the nearest 0.5 mm, photographed with a Fujifilm FinePix 3 digital camera to record color patterns, sampled for genetic analysis, and then preserved as vouchers. Tissue sampling for molecular work involved removing a muscle biopsy, eye, or caudal body portion (depending on size and life stage) and storage in saturated salt buffer (Seutin *et al*. 1990). Genomic DNA was extracted from up to approximately 20 mg of minced preserved tissue via an automated phenol-chloroform extraction on the Autogenprep965 (Autogen, Holliston, MA) using the mouse-tail tissue protocol with a final elution volume of 50 µl. For PCR, 1 µl of this genomic DNA was used in a 10 µl reaction with 0.5U Bioline (BioLine USA, Boston, MA) taq polymerase, 0.4µl 50mM MgCl2, 1µl 10X buffer, 0.5µl of 10mM dNTPs, and 0.3µl of 10µM of each primer FISH-BCL (5¹-TCAACYAATCAYAAAGATATYGGCAC) and FISH-BCH (5¹-TAAACTTCAGGGTGACCAAAAAATCA). Thermal cycler program for PCR was one cycle of 5 m @ 95°C; 35cycles of 30 s @ 95°C, 30 s @ 52°C and 45 s @ 72°C; one cycle of 5 m @ 72°C, and a hold at 10°C. PCR products were purified with Exosap-IT (USB, Cleveland, OH) using 2 µl of 0.2x enzyme and incubated for 30m@37°C. The reaction was then inactivated for 20 m @ 80°C. Sequencing reactions were performed using 1µl of this purified PCR product in a 10 µl reaction containing 0.5 µl primer, 1.75 µl BigDye buffer and 0.5 µl BigDye (ABI, Foster City, CA) and run in the thermal cycler for 30 cycles of 30 s @ 95°C, 30 s @ 50°C, 4 m @ 60°C and then held at 10°C. These sequencing reactions were purified using Millipore Sephadex plates (MAHVN-4550; Millipore, Billerica, MA) per manufacturers instructions and stored dry until analyzed. Sequencing reactions were analyzed on an ABI 3730XL automated DNA sequencer, and sequence trace files were exported into Sequencher 4.7 (GeneCodes, Ann Arbor, MI). Using the Sequencher program, ends were trimmed from the raw sequences until the first and last 10 bases contained fewer than 5 base calls with a confidence score (phred score) lower than 30. After trimming, forward and reverse sequences for each specimen were assembled, each assembled contig was examined and edited by hand, and each sequence was checked for stop codons. Finally the consensus sequence from each contig was aligned and exported in a nexus format. Neighbor-joining trees (Saitou & Nei 1987) and distance matrices were generated using Paup*4.1 (Swofford 2002) on an analysis of Kimura 2-parameter distances (Kimura 1980). Photographs and voucher specimens of each species were then examined to identify distinguishing morphological features for all life-history stages.

Material. *Phaeoptyx* and *Astrapogon* material examined is listed in Table 1. Many of the USNM-cataloged voucher specimens are only partial specimens because of the tissue sample taken for genetic analysis. For all specimens analyzed genetically, a digital color photograph is housed at the Smithsonian Institution and available from the authors. Cytochrome Oxidase 1 sequences for specimens analyzed genetically are deposited in GenBank with accession numbers FJ609838-FJ609940.

TABLE 1. *Phaeoptyx* and *Astrapogon* material. The 4-digit DNA number indicates that the specimen was analyzed for Cytochrome Oxidase 1. If an asterisk appears beside this number, the DNA analysis was successful and the entry appears in the neighbor-joining tree in Fig. 1 or 7. If the specimen was not sampled for DNA, "No DNA" is recorded in this column.

SPECIES	DNA #	SL (mm)	SPECIMEN VOUCHER	PHOTO VOUCHER
Phaeoptyx conklini	5013*	10	No	Yes
Phaeoptyx conklini	5039*	12	No	Yes
Phaeoptyx conklini	5068*	20	USNM 393366	Yes
Phaeoptyx conklini	5069	19	USNM 393367	Yes
Phaeoptyx conklini	5085	30	USNM 393368	Yes
Phaeoptyx conklini	5109*	20	USNM 393369	Yes
Phaeoptyx conklini	5141*	21	USNM 393370	Yes
Phaeoptyx conklini	5142*	20.5	USNM 393371	Yes
Phaeoptyx conklini	5143*	40	USNM 393372	Yes
Phaeoptyx conklini	5144*	22	USNM 393373	Yes
Phaeoptyx conklini	5145*	21	USNM 393374	Yes
Phaeoptyx conklini	5198*	30	USNM 393375	Yes
Phaeoptyx conklini	5303*	30	USNM 393376	Yes
Phaeoptyx conklini	5388*	17	USNM 393377	Yes
Phaeoptyx conklini	5498*	15 - reared	USNM 393378	Yes
Phaeoptyx conklini	6162*	10.5	No	Yes
Phaeoptyx conklini	6372*	10.5	No	Yes
Phaeoptyx conklini	6402*	17	USNM 393381	Yes
Phaeoptyx conklini	6434*	15 - reared	No	Yes
Phaeoptyx conklini	7007*	13.5	USNM 393382	Yes
Phaeoptyx conklini	7011*	15	USNM 393383	Yes
Phaeoptyx conklini	7034	12	USNM 393384	Yes
Phaeoptyx conklini	7094	38	USNM 393385	Yes
Phaeoptyx conklini	7095*	21	USNM 393386	Yes
Phaeoptyx conklini	7254*	10	No	Yes
Phaeoptyx conklini	7255*	11	No	Yes
Phaeoptyx conklini	7278*	13.5	USNM 393390	Yes
Phaeoptyx conklini	7687*	43	USNM 393391	Yes
Phaeoptyx conklini	No DNA	38	USNM 365171	Teeth only
Phaeoptyx pigmentaria	4557*	23 - reared	USNM 393323	Yes
Phaeoptyx pigmentaria	4568*	22 - reared	USNM 393324	Yes
Phaeoptyx pigmentaria	4571*	23 - reared	USNM 393325	Yes
Phaeoptyx pigmentaria	4573*	25 - reared	USNM 393326	Yes
Phaeoptyx pigmentaria	5025*	23	USNM 393332	Yes

Phaeoptyx pigmentaria	5052*	38	USNM 393327	Yes
Phaeoptyx pigmentaria	5053*	27	USNM 393328	Yes
Phaeoptyx pigmentaria	5054*	24	USNM 393329	Yes
Phaeoptyx pigmentaria	5061*	21	USNM 393330	Yes
Phaeoptyx pigmentaria	5062*	21	USNM 393331	Yes
Phaeoptyx pigmentaria	5102*	20	USNM 393333	Yes
Phaeoptyx pigmentaria	5121*	30	USNM 393334	Yes
Phaeoptyx pigmentaria	5122*	25	USNM 393335	Yes
Phaeoptyx pigmentaria	5146*	28	USNM 393336	Yes
Phaeoptyx pigmentaria	5147*	23	USNM 393337	Yes
Phaeoptyx pigmentaria	5269*	30	USNM 393338	Yes
Phaeoptyx pigmentaria	5489*	19 – reared	USNM 393339	Yes
Phaeoptyx pigmentaria	5490*	19 – reared	USNM 393340	Yes
Phaeoptyx pigmentaria	5494*	18 – reared	USNM 393341	Yes
Phaeoptyx pigmentaria	5503*	16	USNM 393342	Yes
Phaeoptyx pigmentaria	5504*	18	USNM 393343	Yes
Phaeoptyx pigmentaria	6144*	25	USNM 393344	Yes
Phaeoptyx pigmentaria	6145*	19.5	USNM 393345	Yes
Phaeoptyx pigmentaria	6202*	14	USNM 393346	Yes
Phaeoptyx pigmentaria	6235*	13	USNM 393354	Yes
Phaeoptyx pigmentaria	6291*	22	USNM 393347	Yes
Phaeoptyx pigmentaria	6299*	31	USNM 393348	Yes
Phaeoptyx pigmentaria	6300*	22	USNM 393349	Yes
Phaeoptyx pigmentaria	6301*	17	No	Yes
Phaeoptyx pigmentaria	6358*	15	USNM 393350	Yes
Phaeoptyx pigmentaria	6370*	30	USNM 393351	Yes
Phaeoptyx pigmentaria	6371*	19	USNM 393352	Yes
Phaeoptyx pigmentaria	6396*	15	No	Yes
Phaeoptyx pigmentaria	6397*	16	No	Yes
Phaeoptyx pigmentaria	6401*	22	USNM 393353	Yes
Phaeoptyx pigmentaria	6448*	19 - reared	USNM 393355	Yes
Phaeoptyx pigmentaria	7013*	15	USNM 393356	Yes
Phaeoptyx pigmentaria	7062*	16.5	USNM 393906	Yes
Phaeoptyx pigmentaria	7080*	15.5	USNM 393358	Yes
Phaeoptyx pigmentaria	7088	15	USNM 393359	Yes
Phaeoptyx pigmentaria	7097*	15	USNM 393361	Yes
Phaeoptyx pigmentaria	7716*	16	USNM 393363	Yes
Phaeoptyx pigmentaria	7742*	11	No	Yes
Phaeoptyx pigmentaria	7823*	14	USNM 394916	Yes
Phaeoptyx pigmentaria	7839*	34	USNM 393364	Yes
Phaeoptyx pigmentaria	No DNA	19	USNM 34718	Teeth only
Phaeoptyx xenus	5465*	28	USNM 393393	Yes
Phaeoptyx xenus	6161*	8	No	Yes
Phaeoptyx xenus	6298*	38	USNM 393395	Yes
Phaeoptyx xenus	6433*	11 - reared	No	Yes

Phaeoptyx xenus	7402	10	No	Yes
Phaeoptyx xenus	7740*	30	USNM 393398	Yes
Phaeoptyx xenus	7741*	21	USNM 393399	Yes
Astrapogon alutus	6013*	5	No	Yes
Astrapogon alutus	6040*	6	No	Yes
Astrapogon alutus	6041*	5	No	Yes
Astrapogon puncticulatus	No DNA	7.5	No	Yes
Astrapogon puncticulatus	No DNA	7.5	No	Yes
Astrapogon puncticulatus	No DNA	9.0	No	Yes
Astrapogon puncticulatus	4172*	13	USNM 393400	Yes
Astrapogon puncticulatus	4449*	9.5	No	Yes
Astrapogon puncticulatus	4540*	9	No	Yes
Astrapogon puncticulatus	4541*	9	No	Yes
Astrapogon puncticulatus	5036*	9	No	Yes
Astrapogon puncticulatus	5127*	10	No	Yes
Astrapogon puncticulatus	5348*	33	USNM 393402	Yes
Astrapogon puncticulatus	5396*	12	USNM 393909	Yes
Astrapogon puncticulatus	5397*	10	No	Yes
Astrapogon puncticulatus	5488*	14 - reared	USNM 393404	Yes
Astrapogon puncticulatus	6086*	14	USNM 393405	Yes
Astrapogon puncticulatus	6435	12 - reared	No	Yes
Astrapogon puncticulatus	6436*	14.5 - reared	No	Yes
Astrapogon puncticulatus	7057	10	No	Yes
Astrapogon puncticulatus	7074*	9.5	No	Yes
Astrapogon puncticulatus	7125*	13	USNM 393407	Yes
Astrapogon puncticulatus	7180	10	No	Yes
Astrapogon puncticulatus	7262*	8.5	No	Yes
Astrapogon puncticulatus	7263*	11.5	USNM 393908	Yes
Astrapogon puncticulatus	7689*	13.5	USNM 393409	Yes
Astrapogon puncticulatus	7784*	10	No	Yes
Astrapogon stellatus	6038*	7	No	Yes
Astrapogon stellatus	6186*	45	USNM 393410	Yes
Astrapogon stellatus	6187*	45	USNM 393411	Yes
Astrapogon stellatus	6188*	28	USNM 393412	Yes
Astrapogon stellatus	6449*	10 - reared	USNM 393413	Yes
Astrapogon stellatus	6450	13 - reared	USNM 393414	Yes
Astrapogon stellatus	No DNA	11 - reared	USNM 394917	Yes
Astrapogon stellatus	No DNA	12 - reared	USNM 394917	Yes
Astrapogon stellatus	7291	42	USNM 393415	Yes
Astrapogon stellatus	7292	37	USNM 393416	Yes
Astrapogon stellatus	7294*	46	USNM 393418	Yes
Astrapogon stellatus	7295*	44	USNM 393419	Yes
Astrapogon stellatus	7296*	50	USNM 393420	Yes
Astrapogon sp. (not *A. puncticulatus*)	No DNA	7 specimens, all 6 mm	USNM 394918	No

Phaeoptyx

Identification. Our *Phaeoptyx* specimens form three distinct genetic lineages, corresponding to the three known species (Fig. 1). Intraspecific variation ranges from 0.00–2.19% nucleotide substitutions per site as opposed to 15.20–20.80% for interspecific variation (Table 2). See "Comments on Identification of Adult *Phaeoptyx*," below, for a discussion of morphological identification of adult *Phaeoptyx*.

TABLE 2. Average (and range) Kimura 2-parameter distance summary for *Phaeoptyx* species. Intraspecific averages are in bold font.

	P. pigmentaria N=44	*P. conklini* N=26	*P. xenus* N=6
P. pigmentaria	**0.56% (0.00–2.19)**		
P. conklini	16.79% (15.65–19.02)	**0.78% (0.00–5.81)**	
P. xenus	16.03% (15.20–20.80)	18.10% (16.71–20.80)	**0.43% (0.00–0.93)**

Larvae (Fig. 2). *Phaeoptyx* larvae are easily distinguished from other apogonids by the presence of yellow chromatophores on the first dorsal and pelvic fins and usually melanophores on some portion of the pelvic fin. The yellow fin pigment typically disappears about the same time as the blotch of melanophores appears on the caudal peduncle, and here we separate larval and juvenile stages based on this transition in pigment. This also corresponds well with settlement from the plankton, as most of our net-collected larvae that have caudal-peduncle pigment do not have the pigment well defined as it is in juveniles and adults. Diagnostic features of the species are given below.

Phaeoptyx conklini: Size range 10–13.5 mm SL. First dorsal fin yellow; no melanophores. Second dorsal fin clear. Pelvic fin yellow, usually more strongly so proximally, with a prominent melanophore at the base and linear series of melanophores along the fin rays to the distal tip of the fin. Anal fin mostly clear; orange, if present, restricted to base. Posterior end of caudal peduncle with two dense, vertical crescent-shaped bands of orange chromatophores overlying posterior margin of hypural plate, the dorsal one only partially formed in small specimens. Caudal fin with orange chromatophores on bases of central rays; remainder of fin without pigment. Head mostly orange, some specimens with yellow chromatophores over posterior portion of brain; scattered melanophores present on top of head, a couple sometimes present on cheek, and heavy pigment developing in temporal region by approximately 12 mm. Scattered melanophores present on gut and a cap of melanophores usually visible over swimbladder in all larvae, and a series of internal melanophores usually developing along anterior portion of vertebral column by 12 mm.

Phaeoptyx pigmentaria: Size range 13–18 mm SL. First dorsal fin usually yellow, last two elements sometimes orange; melanophores, if present, restricted to the distal half of anterior spines. Second dorsal fin with orange stripe along proximal one fifth to one third of fin rays. Pelvic fin yellow; melanophores, if present, restricted to distal half of fin (or distal tips of fin rays). Anal fin mostly orange. Posterior end of caudal peduncle with two dense, vertical crescent-shaped bands of orange chromatophores overlying posterior margin of hypural plate; caudal fin mostly orange, melanophores usually present at distal ends of outer branched rays of dorsal and ventral lobes. Head mostly orange, some specimens with yellow chromatophores over posterior portion of brain; scattered melanophores present on top of head and a few present on cheek/temporal region by 13 mm; larger larvae usually also with melanophores on distal margins of premaxilla and dentary. Scattered melanophores present on gut, and a cap of melanophores visible over swimbladder in all larvae; a series of internal melanophores developing along anterior portion of vertebral column by 15 mm.

Phaeoptyx xenus: Size range 8–10 mm SL. First dorsal fin primarily yellow, last two fin elements clear; no melanophores. Second dorsal-fin spine elongate, extending when depressed beyond posterior base of second dorsal fin in 8-mm SL specimen, to middle of second dorsal fin in 10-mm SL specimen. Second dorsal fin clear. Pelvic fin orange at base; the remainder of fin yellow with scattered melanophores on the distal

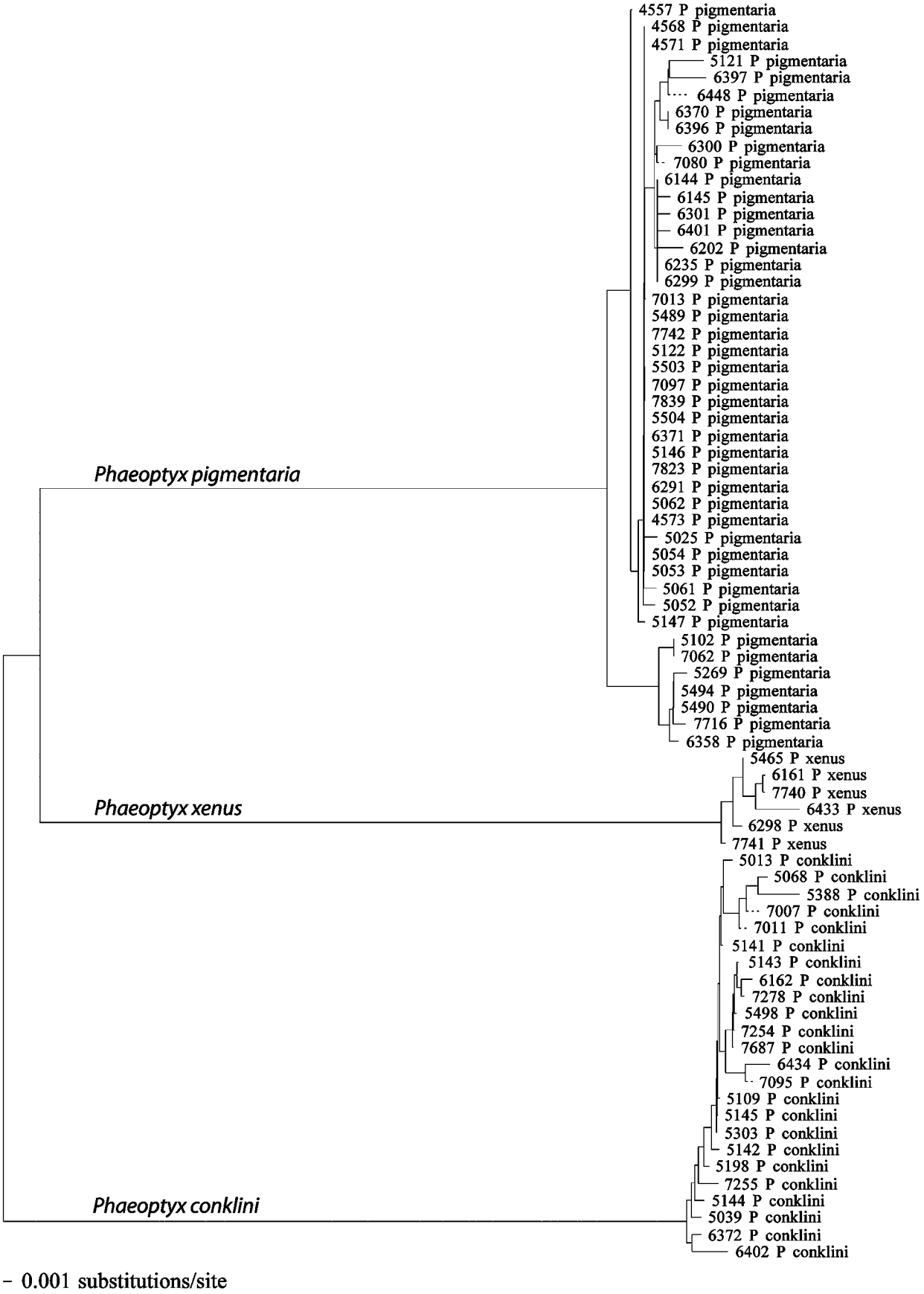

FIGURE 1. Neighbor-joining tree derived from Cytochrome Oxidase 1 sequences showing three genetically distinct lineages of Belizean *Phaeoptyx*.

FIGURE 2. *Phaeoptyx* larvae: (A) *Phaeoptyx conklini*, 12 mm SL, 5039; (B)*Phaeoptyx pigmentaria*, USNM 393356, 15 mm SL, 7013, (C) *Phaeoptyx pigmentaria*, USNM 393358, 15.5 mm SL, 7080; (D) *Phaeoptyx xenus*, 8 mm SL, 6161.

three quarters. Anal fin primarily clear. Posterior end of caudal peduncle with two vertical crescent-shaped bands of orange chromatophores overlying posterior margin of hypural plate, the dorsal one only partially formed in 10 mm specimen, absent in 8 mm specimen. Caudal fin with a few scattered chromatophores on anterior portion of central rays; remainder of fin clear. Head mostly orange, with bright yellow chromatophores over posterior portion of brain; a few melanophores present on top of head and two to several present on temporal region. Lateral surface of gut yellow in 8-mm specimen, pale yellow and with a few melanophores in 10 mm specimen. A cap of melanophores developing over swimbladder in 8 mm specimen, well developed and prominent at 10 mm. The 10 mm specimen also with a well-developed series of internal melanophores on anterior portion of vertebral column.

Comparisons (Table 3): The patterns of chromatophores and melanophores on the fins usually distinguish larvae of the three species. *Phaeoptyx pigmentaria* is unique in typically having melanophores on the first dorsal and caudal fins, a wide orange stripe at the base of the second dorsal fin and a mostly orange caudal fin. *Phaeoptyx conklini* is distinctive in having a prominent melanophore at the base of (and usually spots along the entire length of) the pelvic fin; in the other species, melanophores are restricted to the distal half (*P. pigmentaria*, if present) to three quarters (*P. xenus*) of the fin. *Phaeoptyx xenus* is distinctive in having the second dorsal-fin spine elongate. Some of the differences noted above may be related to developmental stage and size: although *P. pigmentaria* is the most common larval *Phaeoptyx* species in our samples, we have not obtained larvae smaller than 13 mm, whereas the largest larvae of *P. xenus* and *P. conklini* we have collected are 10 and 13.5 mm, respectively.

TABLE 3. Diagnostic characters of *Phaeoptyx* larvae from Belize, Central America.

Species	Second D_1 Element Elongate	Melanophores: D_1	Melanophores: P_2	Melanophores: Caudal Fin
P. conklini 10–13.5 mm SL	No	No	Yes, at base and along entire length of fin	No
P. pigmentaria 13–18 mm SL	No	Usually several near distal tips of anterior spines	Yes, on distal ½ of fin or restricted to distal tips of rays	Yes, numerous on distal tips of outer branched rays
P. xenus 8–10 mm SL	Yes	No	Yes, on distal ¾ of fin	No

Species	Chromatophores: D_1	Chromatophores: D_2	Chromatophores: Caudal Fin
P. conklini 10-13.5 mm SL	Yes, orange on last element or two	No distinct stripe	Yes, a few orange spots near bases of central rays
P. pigmentaria 13-18 mm SL	Yes, orange on last element or two	Yes, distinct orange stripe at base	Yes, orange covering most of fin
P. xenus 8-10 mm SL	No	No distinct stripe	Yes, a few orange spots near bases of central rays

Juveniles (Fig. 3). *Phaeoptyx* juveniles can be distinguished from other juvenile apogonids by the pattern of pigment – from *Astrapogon* in lacking heavily pigmented first dorsal and pelvic fins, and from *Apogon* in always having a blotch of pigment on the caudal peduncle (vs. caudal-peduncle spot present only in some species), trunk beginning to develop uniform covering of melanophores (vs. melanophores only in discrete spots or bars), and in having a pale pink color (vs. having bright orange chromatophores covering most of trunk).

FIGURE 3. *Phaeoptyx* juveniles: (A) *Phaeoptyx conklini*, USNM 393373, 22 mm SL, 5144; (B) *Phaeoptyx pigmentaria*, USNM 393352, 19 mm SL, 6371; (C) *Phaeoptyx xenus*, USNM 393399, 21 mm SL, 7741.

Phaeoptyx conklini: Size range 14–22 mm SL. Snout and upper and lower jaws with orange chromatophores, never yellow. Tip of snout blunt, forming almost a 90º angle with dorsal profile. Second dorsal and anal fins lacking distinct stripes of orange pigment but entire fin may be orange. Specimens 15 mm SL and smaller lacking melanophores on second dorsal and anal fins. Specimens 17 mm SL and larger with

distinct stripes of melanophores forming at bases or slightly apart from bases of second dorsal and anal fins. Posterior end of caudal peduncle with vertical, dark bar spanning most of peduncle; anterior edge of bar irregular, the central portion usually extending further anteriorly than rest of bar. Posterior end of peduncle retaining the two vertical crescent-shaped bands of orange chromatophores of larvae. Caudal fin orange and with numerous melanophores in wildcaught juveniles, usually pale and with fewer melanophores in reared specimens. Teeth, when developed, small (see Fig. 5).

Phaeoptyx pigmentaria: Size range 17–21 mm SL. Snout and upper and lower jaws usually with yellow chromatophores. Tip of snout rounded. No stripe of melanophores along bases of second dorsal and anal fins. Second dorsal fin with orange stripe along proximal one fifth to one third of fin rays. Anal fin with similar orange stripe in wildcaught specimens (lacking in some reared juveniles). Caudal peduncle with dark bar usually spanning entire peduncle; anterior edge of bar relatively straight and uniform. Posterior end of peduncle retaining the two vertical crescent-shaped bands of orange chromatophores of larvae. Caudal fin mostly pale orange, with numerous scattered melanophores. Teeth, when developed, enlarged (see Fig. 5).

Phaeoptyx xenus: Presumably juveniles are typically larger than our largest larva, 10 mm SL, and smaller than our smallest adult, 28 mm SL; our description here is based on one reared larva measuring 11 mm SL and a wild-caught juvenile of 21 mm SL. Snout and upper and lower jaws pale yellow; tip of snout rounded. Second dorsal and anal fins lacking distinct stripes of pigment in 11 mm specimen, with well-developed stripes of pigment in 21 mm specimen. First dorsal and caudal fins with scattered melanophores in 21 mm specimen. Center of caudal peduncle with vertical oval of melanophores, oval not reaching dorsal and ventral body margins. Posterior end of caudal peduncle with two crescent-shaped lines of orange chromatophores as in larvae. Teeth small.

TABLE 4. Diagnostic characters of *Phaeoptyx* juveniles from Belize, Central America.

Species	Yellow Snout and Jaws	Tip of Snout	Enlarged Teeth in Jaws	Melanophores: Stripes at Bases of D_2 and A
P. conklini 14-22 mm SL	No	Blunt	No	Developing; pale streak between stripe and base of fin
P. pigmentaria 17-21 mm SL	Yes	Rounded	Yes, when developed	No
P. xenus 11, 21 mm SL	Yes	Rounded	No	Developing; no pale streak between stripe and base of fin

Species	Melanophores: Caudal Peduncle	Chromatophores: D_2	P_1 Rays
P. conklini 14-22 mm SL	Bar with irregular anterior margin, usually extending to dorsal and ventral body margins	No stripe; entire fin may be pale orange	11–13
P. pigmentaria 17-21 mm SL	Bar with regular anterior margin, usually extending to dorsal and ventral body margins	Proximal orange stripe	11–13
P. xenus 11, 21 mm SL	Central oval, not extending to dorsal and ventral body margins	No	10

Comparisons (Table 4)**:** Juveniles typically can be separated by the following: size and shape of the blotch of pigment on the caudal peduncle (spanning entire depth of peduncle and with a relatively uniform anterior margin in *P. pigmentaria,* usually spanning entire depth of peduncle and with irregular anterior margin in *P. conklini,* more oval shaped and not reaching dorsal and ventral body margins in *P. xenus*); presence or absence of yellow pigment on the snout and tip of lower jaw (usually present in *P. pigmentaria* and *P. xenus,* absent in *P. conklini*); shape of the tip of the snout (blunt in *P. conklini,* rounded in *P. pigmentaria* and *P. xenus*); presence or absence of basal stripes of melanophores on the second dorsal and anal fins (absent in *P. pigmentaria,* present and set slightly apart from bases of fins in *P. conklini,* present and covering bases of fins in *P. xenus*); and presence or absence of orange pigment proximally on second dorsal

(and usually anal) fin (present in *P. pigmentaria*, absent in *P. conklini* and *P. xenus)*. Larger juveniles of *P. pigmentaria* differ from the others in having enlarged teeth. Additionally, *Phaeoptyx xenus*, which is not common in our samples at any life-history stage, has 10 pectoral-fin rays vs. 11–13 in the other two species.

Comments on Identification of Adult *Phaeoptyx* (Fig. 4, Table 5). Adult *P. pigmentaria* is easily identified by the absence of a well-developed pigment stripe along the bases of the second dorsal and anal fins (although there may be a small stripe comprising one or two rows of melanophores as well as numerous scattered spots on both fins – Fig. 4D); presence of enlarged teeth in the upper and lower jaws (Fig. 5); and by having 11–13 (rarely 13) lower-limb gill rakers (vs. 13–15, usually 14, in *P. xenus*, 14–16, usually 15, in *P.* conklini—Böhlke and Chaplin 1968). *Phaeoptyx conklini* has traditionally been separated from *P. xenus* by the presence of wide, dark stripes of pigment along the bases of the second dorsal and anal fins, a larger eye, and modally one more gill raker (15 vs. 14) on the lower limb (Böhlke & Chaplin 1968; Gon 2002). There is overlap in extremes of eye diameter (Fig. 6), the stripe of fin pigment may be wider in *P. xenus* than in *P. conklini*, and accurate gill-raker counts are sometimes difficult to make in small adults, especially in the field. As noted by Böhlke and Chaplin (1968), the fin stripes in *P. conklini* are usually darker and more prominent than in *P. xenus* and set slightly apart from the bases vs. directly along the bases. However, the intensity of the stripes is most informative when both species are available for comparison, and several of our *P. conklini* adults lack the pale streaks or gaps between the stripes of fin pigment and fin bases. The stripe of pigment on the anal fin does not extend anteriorly to the first and second anal-fin elements in *P. xenus*, whereas it does extend to the first two elements in *P. conklini.* Additionally, adult *P. conklini* retain the very blunt snout of juveniles, and it is not yellow as it is in adult *P. pigmentaria* and *P. xenus*. If the mouth is closed, the morphology of the snout is often the most distinctive diagnostic character of *P. conklini*. *Phaeoptyx conklini* usually has the pigment spot on the caudal peduncle extending to or near the dorsal and ventral body margins, whereas this spot is typically restricted to the center of the caudal peduncle in *P. xenus.* Finally, adults of all three species can usually be separated by the size, number, and distribution of melanophores on the trunk: in *P. pigmentaria*, there is usually one melanophore per scale, and the pigment forms uniform rows of spots from anterior to posterior (Fig. 4C); in *P. conklini*, there are usually two or more melanophores per scale posteriorly, and the pigment spots are uniformly spaced on the trunk (Fig. 4B); in *P. xenus*, the trunk pigment is irregular, with larger melanophores scattered along the center and smaller, more densely clustered melanophores dorsally and ventrally (Fig. 4E, F). As with most other characters of *Phaeoptyx*, however, there is variation within species, and one specimen of *P. pigmentaria*, for example, has a pattern of trunk melanophores more like that of *P. xenus* than other *P. pigmentaria* (Fig. 4D).

TABLE 5. Diagnostic characters of *Phaeoptyx* adults from Belize, Central America.

Species	Yellow Snout	Tip of Snout	Enlarged Teeth	Melanophores D_2 and A	Melanophores Trunk	Melanophores Caudal Peduncle	Lower-Limb Gill Rakers
P. conklini > 22 mm SL	No	Blunt	No	Well-developed dark stripe; usually a pale streak between stripe and base of fin	Multiple per scale; spots usually uniformly spaced	Bar usually spanning entire width; anterior margin usually irregular	14–16, usually 15
P. pigmentaria > 21 mm SL	Yes	Rounded	Yes	No well-developed stripe; may be a single or double row of melanophores and others scattered across fin	One per scale, occasionally two; spots usually uniformly spaced	Bar spanning entire width; anterior margin typically uniform.	11–13, usually 11 or 12
P. xenus > 21 mm SL	Yes	Rounded	No	Well-developed stripe but usually not dark; no pale streak between stripe and base of fin	Multiple per scale; spots irregularly spaced; larger spots in center, smaller spots at periphery	Vertical oval, not reaching dorsal and ventral margins	13 –15, usually 14

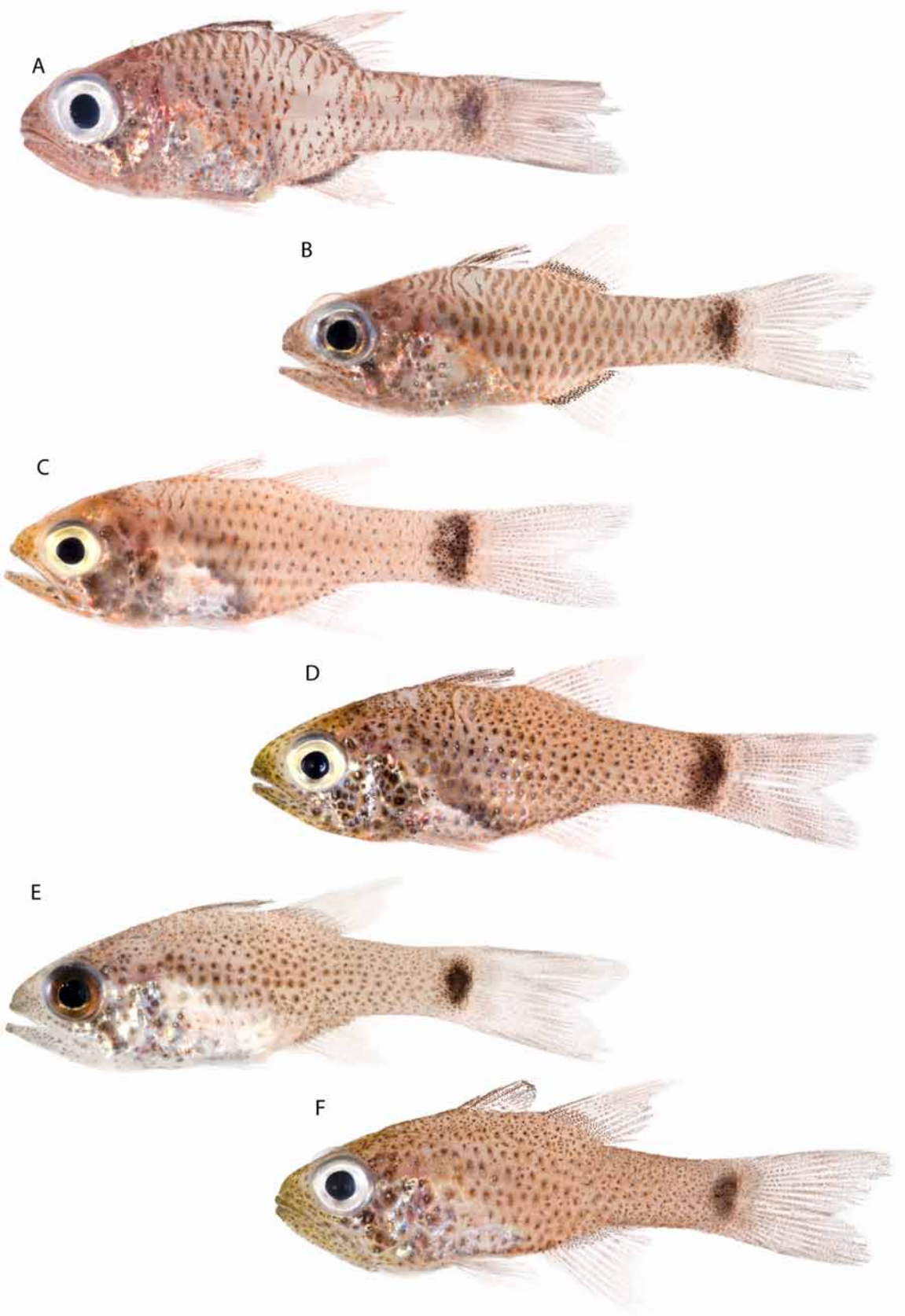

FIGURE 4. *Phaeoptyx* adults: (A) *Phaeoptyx conklini*, USNM 393372, 40 mm SL, 5143; (B) *Phaeoptyx conklini*, USNM 393376, 30 mm SL, 5303; (C) *Phaeoptyx pigmentaria*, USNM 393338, 30 mm SL, 5269; (D) *Phaeoptyx pigmentaria*, USNM 393327, 38 mm SL, 5052; (E) *Phaeoptyx xenus*, USNM 393395, 38 mm SL, 6298; (F) *Phaeoptyx xenus*, USNM 393393, 28 mm SL, 5465.

FIGURE 5. Teeth of *Phaeoptyx conklini* (A), USNM 365171, 38 mm SL, and *Phaeoptyx pigmentaria* (B), USNM 347318, 19 mm SL.

Comments on Previous Identifications of Early Life Stages of *Phaeoptyx*. Lara (2006:1393) provided illustrations of a 12 mm larva and a 16.2 mm late larval or juvenile specimen of *P. conklini*. The larva has the hallmark *P. conklini* pelvic-fin pigment—melanophores along the entire length of the fin including a spot at the base. In the description of larvae, however, Lara (2006:1392) did not mention pelvic-fin pigment. The 16.2-mm specimen exhibits the blunt snout, small teeth, and caudal-peduncle pigment spanning entire depth of peduncle and with irregular anterior edge typical of juvenile *P. conklini*. Lara (2006:1395) provided an illustration of a 17.2-mm SL specimen of *P. pigmentaria* that resembles our juvenile specimens of that species in having the blotch of melanophores on the caudal peduncle extending the entire depth of the peduncle and lacking a blunt snout, although the snout in that illustration is considerably more pointed than the snout is in any of our larval, juvenile, and adult specimens. Lara (2006:1394) noted that larval *P. pigmentaria* has little or no pigment on the fins, but most of our specimens have diagnostic melanophores at the distal tips of the first dorsal, pelvic, and caudal fins. Lara (2006: 1397) illustrated 13.9- and 22.1-mm SL specimens of *P. xenus*. The configuration of the blotch of pigment on the caudal peduncle, which, in both specimens does not extend to the dorsal and ventral margins of the body, agrees with our juveniles of *P. xenus*. The pelvic fin of the 13.9 mm specimen lacks the pigment characteristic of our smaller *P. xenus* larvae. One additional illustration of *Phaeoptyx* provided by Lara (2006:1399:Figure 3C) could be *P. pigmentaria* based on the absence of pelvic-fin pigment.

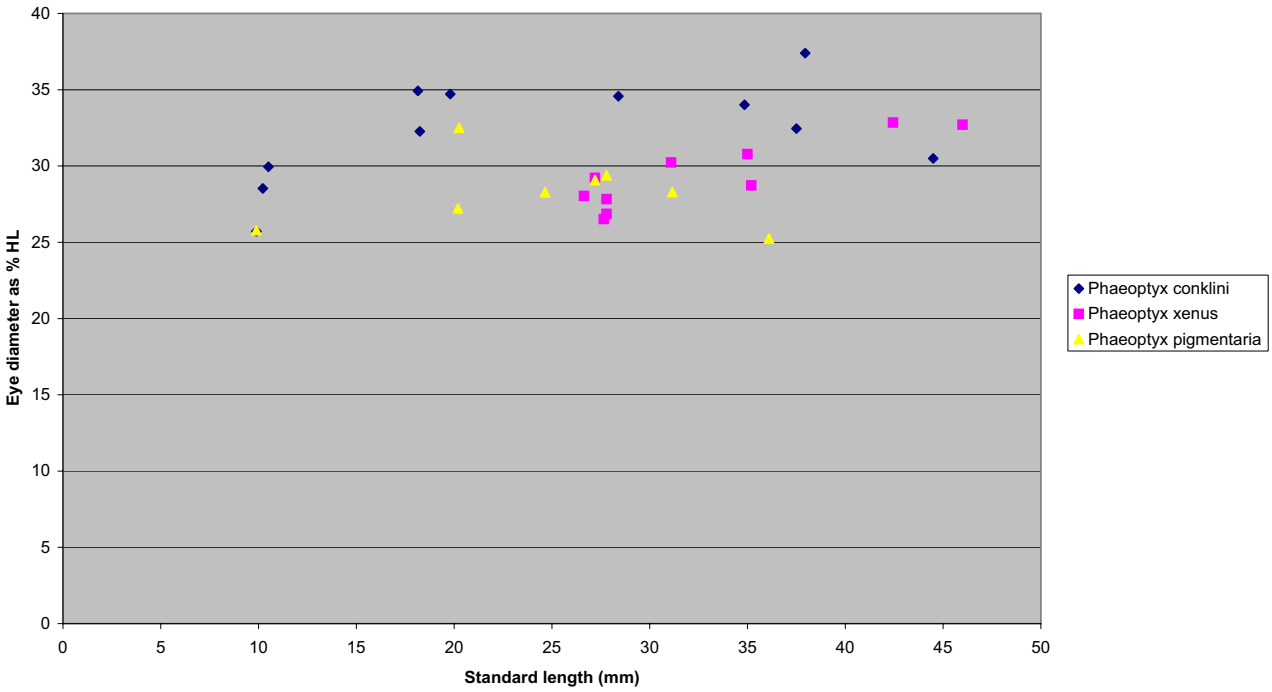

FIGURE 6. Comparison of eye diameter among adult *Phaeoptyx*.

Astrapogon

Identification. *Astrapogon* specimens in our samples form 3 distinct genetic lineages corresponding to the three known species (Fig. 7). Intraspecific variation ranges from 0.00–1.30% nucleotide substitutions per site as opposed to 15.62–20.06% for interspecific variation (Table 6). We identified adult *A. puncticulatus* and *A. stellatus* through counts of pectoral-fin rays and gill rakers in adults (*A. puncticulatus* typically with 16 pectoral-fin rays and 12–13 lower-limb gillrakers; *A. stellatus* usually with 15 pectoral-fin rays and 11 lower-limb gill rakers—Böhlke & Randall 1968). By process of elimination, the third clade, comprising only larvae, is *A. alutus*.

TABLE 6. Average (and range) Kimura 2-parameter distance summary for *Astrapogon* species. Intraspecific averages are in bold font.

	A. alutus N=3	*A. puncticulatus* N=18	*A. stellatus* N=7
A. alutus	**0.72%** (0.62–0.93)		
A. puncticulatus	19.11% (18.44–20.06)	**0.39%** (0.00–1.30)	
A. stellatus	16.06% (15.62–16.62)	17.13% (16.60–18.23)	**0.29%** (0.00–0.62)

Larvae and Juveniles (Figs. 8, 9). *Astrapogon* larvae can be distinguished from larvae of other apogonids by the heavily pigmented (often black) first dorsal and pelvic fins, and usually by the trunk melanophores, which, when expanded, cover most of the body and variously obscure the pale orange chromatophores beneath. (Note: reared juvenile specimens are available for *A. puncticulatus* and *A. stellatus* but not for *A. alutus;* we incorporate notes on the juveniles within the appropriate sections below.)

Astrapogon alutus: Three larvae, 5–6 mm SL. First dorsal and pelvic fins heavily pigmented, with large dark spots (interradial membranes of first dorsal fin almost black in one 5-mm SL specimen) and yellow chromatophores. Second dorsal and anal fins mostly clear, one or two melanophores extending from trunk onto bases of fins. Caudal fin with no pigment in one 5 mm specimen, with one orange chromatophore at base of one ray in dorsal lobe in the other 5 mm specimen, and with several orange chromatophores at bases of central and ventral rays in 6 mm specimen. Posterior end of caudal peduncle clear, trunk pigment ending abruptly on anterior part of peduncle. Background color of head and body yellow and orange. Large melanophores present on head and body. Snout, jaws, cheek, temporal region, and lateral surface of gut with melanophores. A dense cap of melanophores visible over swimbladder.

Astrapogon puncticulatus: Size range of larvae 7.5–12 mm SL; size range of juveniles 10–14.5 mm SL. First dorsal and pelvic fins heavily pigmented in larvae, almost black but with some yellow pigment on or between fin elements. Second dorsal fin mostly clear, one or two melanophores usually extending from trunk onto base of fin. Anal fin primarily clear, a melanophore usually extending from trunk onto base of fin and one melanophore usually present at base of anterior part of fin—sometimes on one side of body only. Caudal fin usually with a few orange chromatophores at bases of central rays. In reared specimens 12 mm SL and larger, heavy pigment present on second dorsal and anal fins, but both fins with distinctive clear areas; second dorsal usually without pigment along distal edges of all rays, posteriormost one or two rays, and near proximal base of posterior fin rays; these clear areas roughly forming a semicircle around pigmented portion; anal fin with similar clear areas in most specimens. Caudal fin in reared specimens mostly dark; distal tips of all rays and proximal portions of central (and sometimes additional) rays clear. In larvae, posterior end of caudal peduncle with three melanophores in vertical bar. In one 9-mm SL larva this bar of melanophores separated by a small but distinct gap from the pigment immediately anterior to it; in another 9 mm specimen this small gap present on left side of body only. Background color of head and body mostly orange, some yellow pigment above swimbladder. Large melanophores present on head, usually including top of head, snout, jaws, cheek, and temporal region; head pigment sometimes including four conspicuous lines of pigment radiating from eye—one dorsally, two posteriorly, and one ventrally. Large melanophores present on body but conspicuously absent on dorsal trunk just anterior to first dorsal fin, just behind second dorsal fin, and posterior to gut; when trunk melanophores in expanded state, these areas conspicuously paler than remainder of trunk. In one of smallest larvae available, 8.5 mm, trunk lacking melanophores except two beneath first dorsal fin and one on caudal peduncle. In a 7.5-mm specimen, trunk melanophores contracted but present in typical number and pattern for *A. puncticulatus.* Reared juveniles usually darker in overall body color than larvae and exhibiting less orange coloration; pale areas as described above still evident in most juveniles. Lines of pigment radiating from eye observed in larvae usually visible in juveniles. Cap of melanophores usually visible over gut and swimbladder as in larvae.

Astrapogon stellatus: One larva, 7 mm SL; four juveniles reared from wildcaught larvae, 10–13 mm SL. In the larva, first dorsal and pelvic fins heavily pigmented, almost black but with some yellow pigment on or

between fin elements. Second dorsal and anal fins mostly clear, trunk melanophores extending onto base of fin. In reared specimens, second dorsal and anal fins each with a blotch of melanophores set within and usually entirely surrounded by pale remainder of fin. In specimens with greatly expanded melanophores, basal fin pigment sometimes merging slightly with the blotch of melanophores on fin. Caudal fin without melanophores in larva but with a few scattered orange chromatophores at bases of central and ventral rays. In reared specimens caudal fin with two large, rounded melanophores at base of fin and one or two smaller melanophores above upper spot and below lower; fin rays clear centrally; two concentrations of melanophores present posteriorly (appearing either as two patches of small spots or as two large pigmented blotches), one on upper caudal lobe and one on lower. Tips of fin rays clear. Background color of head and body mostly orange in larval specimen, some yellow anteriorly; no chromatophores on body in reared specimens. Large, greatly expanded melanophores covering head and body in larva. Trunk nearly chocolate brown in reared specimens, with expanded but distinct melanophores in other two reared specimens. Posterior end of caudal peduncle in larva with at least two melanophores in vertical bar and with a clear area anterior to those spots. Caudal peduncle uniformly dark in chocolate brown reared specimens, with small clear area anterior to bar of melanophores on posterior end of caudal peduncle in other reared specimens. Snout, jaws, cheek, temporal region, and lateral surface of gut with melanophores. A dense cap of melanophores visible over gut and swimbladder in larva.

Comparisons: Larvae of *A. puncticulatus* differ from the other species in usually having three areas on the trunk that conspicuously lack melanophores: just anterior to origin of first dorsal fin, just posterior to second dorsal fin, and just above anterior end of anal fin (just posterior to the gut). These gaps can be seen in specimens in which the trunk melanophores are contracted (Fig. 8E) or expanded (Fig. 8D). The gap behind the second dorsal fin is sometimes small (Fig. 8F) because of an additional melanophore on the dorsal portion of the caudal peduncle, and in one unusual specimen (Fig. 8G), so few melanophores are present that there is no conspicuous pattern of gaps. Larvae of *A. stellatus* sometimes exhibit less distinctive pale areas in similar but slightly different locations than those in *A. puncticulatus* (Fig. 8C): the gap anterior to origin of first dorsal fin is a bit more forward, the gap at posterior end of second dorsal fin is below the fin (vs. behind it), and the gap above anal fin is above the anterior third of the fin (vs. above the most anterior point of the fin). Larvae of *A. puncticulatus* have an overall paler appearance than larvae of *A. alutus* and *A. stellatus;* this is, in part, because the pale areas described above reveal more of the pale orange background body color but also because the trunk melanophores, even when expanded, are generally smaller or lighter than those in the other species. Most *A. puncticulatus* larvae lack a clear area on the caudal peduncle anterior to the posterior bar of melanophores, a feature present in the larval *A. stellatus*; the entire peduncle is clear in 5- and 6-mm SL specimens of *A. alutus*. A small clear gap is present on the caudal peduncle in two 9-mm SL specimens of *A. puncticulatus,* however, suggesting that it could be present in early ontogenetic stages of all three species.

It is not possible with our limited larval *Astrapogon* material to definitively diagnose larvae of *A. alutus* and *A. stellatus. Astrapogon alutus* differs from the other species in having yellow and orange body coloration vs. primarily orange. However, the largest (and only) larvae of *A. alutus* we have collected are 5 and 6 mm SL, and the smallest larvae of *A. puncticulatus* and *A. stellatus* are 8.5 and 7 mm SL, respectively. Accordingly, the differences in body coloration could be attributable to differences in size or developmental stage.

Astrapogon alutus is unique in having no melanophores on the caudal peduncle, and *A. stellatus* has a bar of melanophores posteriorly on the caudal peduncle preceded by a clear area. Those differences may reflect variation typical of early larval stages: in a series of *Astrapogon* larvae from a single collection (all approximately 6 mm SL) not identified using DNA but representing either *A. alutus* or *A. stellatus* based on trunk pigmentation, the pigment on the caudal peduncle is highly variable: pigment is absent on the caudal peduncle in one specimen, bears a single spot on one side and several on the other in another specimen, has a posterior bar of pigment and a clear area preceding it in several specimens, and has the peduncle entirely pigmented in another.

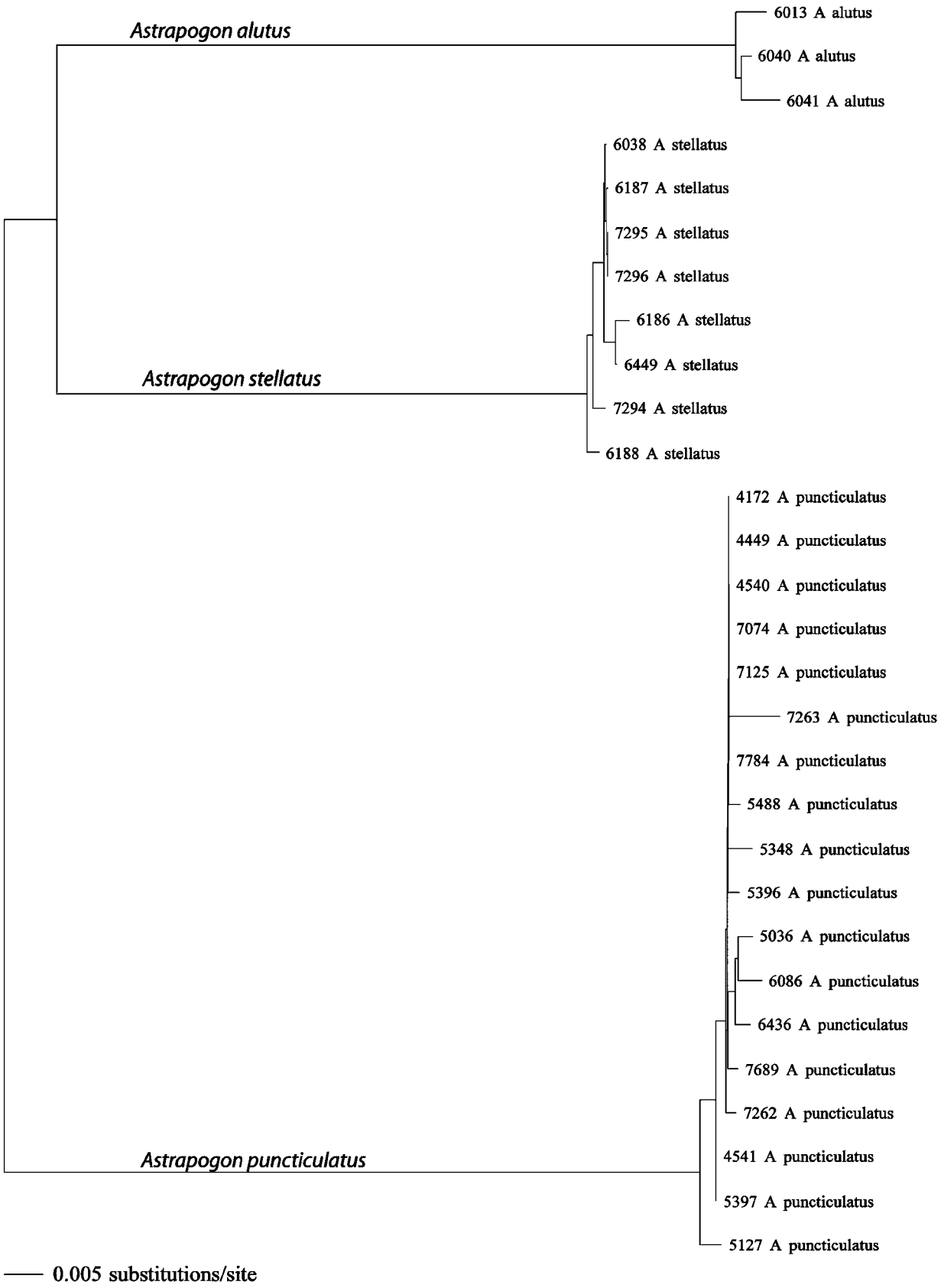

FIGURE 7. Neighbor-joining tree derived from Cytochrome Oxidase 1 sequences showing three genetically distinct lineages of Belizean *Astrapogon.*

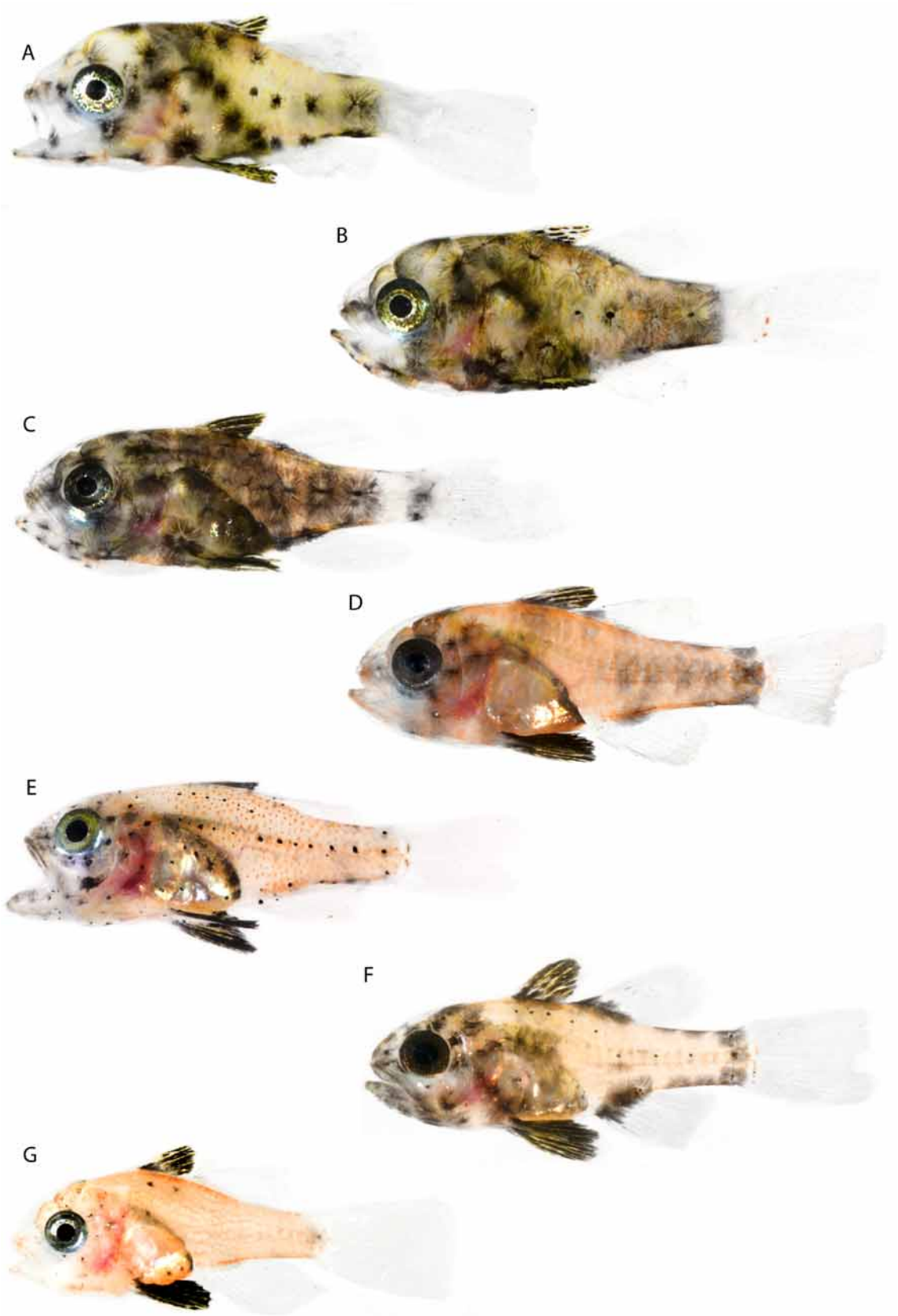

FIGURE 8. *Astrapogon* larvae: (A) *Astrapogon alutus*, 5 mm SL, 6041; (B) *Astrapogon alutus*, 6 mm SL, 6040; (C) *Astrapogon stellatus*, 7 mm SL, 6038; (D) *Astrapogon puncticulatus*, 9.5 mm SL, 4449; (E) *Astrapogon puncticulatus*, USNM 393909, 12 mm SL, 5396; (F) *Astrapagon puncticulatus*, USNM 393407, 13 mm SL, 7125; (G) *Astrapagon puncticulatus*, 8.5mm SL, 7262.

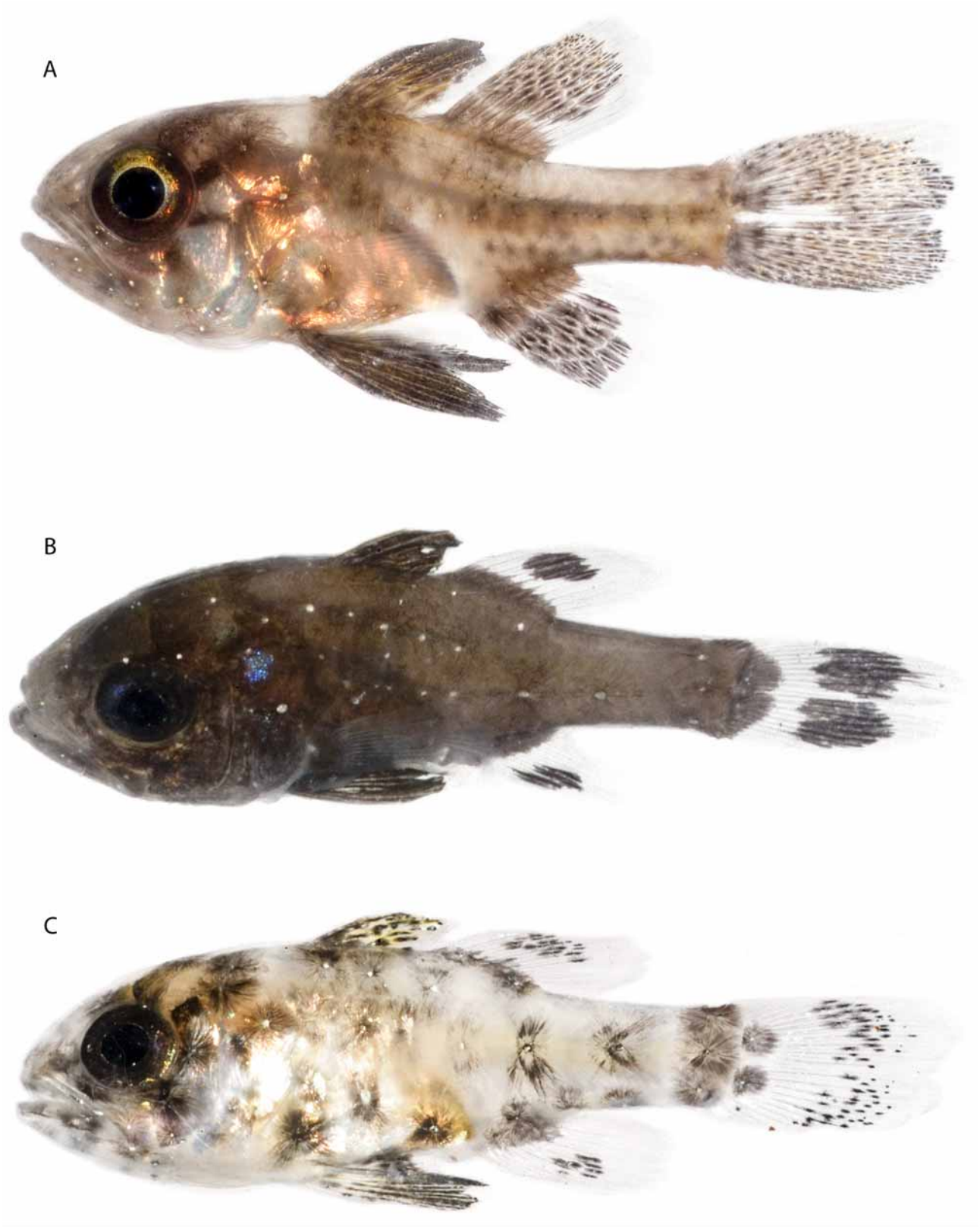

FIGURE 9. *Astrapogon* juveniles: (A) *Astrapogon puncticulatus*, USNM 393404, 14 mm SL, 5488, reared specimen; (B) *Astrapogon stellatus*, USNM 393413, 10 mm SL, 6449, reared specimen; (C) *Astrapogon stellatus*, USNM 393413, 13 mm SL, 6450, reared specimen.

Juveniles of *A. puncticulatus* differ from those of *A. stellatus* in the patterns of pigment on the second dorsal, anal, and caudal fins: in *A. puncticulatus*, the second dorsal and anal fins have a large blotch of pigment with clear areas forming roughly a semicircle around the posterior end of the blotch; in *A. stellatus*, the pigment blotch on the second dorsal and anal fins is smaller and completely surrounded by clear areas. Caudal-fin pigment of juvenile *A. puncticulatus* comprises a horizontally elongate oval centered on the proximal portion of the fin, whereas in *A. stellatus* the caudal fin has two large, rounded melanophores at the base of the fin and two concentrations of melanophores further out on the fin. Identification of juvenile *A. alutus* is needed to determine if the fin pigment in *A. puncticulatus* or *A. stellatus*, or both, is diagnostic.

Comments on previous identifications of *Astrapogon* larvae. Lara (2006:1391) provided illustrations of two specimens of *A. stellatus*, 5.7 and 7.8 mm SL. The 5.7 mm specimen looks very similar to both our 6 mm specimen of *A. alutus* and 7 mm specimen of *A. stellatus* except that it lacks a clear area on the caudal peduncle. Lara's 7.8 mm specimen of *A. stellatus* bears the distinctive pale areas in front of the first dorsal fin, beneath the posterior portion of the second dorsal fin, and above the anterior third of the anal fin characteristic of some of our larval *A. stellatus*. As noted above, *A. puncticulatus* has similar pale areas, but it is never as uniformly covered with melanophores as in Lara's specimen and our *A. stellatus*. Lara (2006:1389) illustrated five larval and juvenile specimens of *A. puncticulatus*. The pale areas characteristic of *A. puncticulatus* appear to be present in the 5.6, 8.6, and 14.3 mm specimens (her figures A, B, D). The 10.5 mm larva (her figure C) appears to have small melanophores anterior to the first dorsal fin and several melanophores behind the second dorsal fin. The absence in that specimen of pigment on the first dorsal and pelvic fins is odd and unlike that of any larval *Astrapogon* specimen we have examined. Possibly there is geographical variation in pigment patterns of *Astrapogon* between Florida (where Lara's specimens were obtained) and Belize. The 16.8 mm juvenile (her figure E) appears to have the clear area on the proximal part of the posterior half of the second dorsal fin typical of *A. puncticulatus* and a similar band on the anal fin.

Lara (2006) did not indicate how she identified the larval stages of *Astrapogon* prior to their developing the full complement of gill rakers and pectoral-fin rays, and her descriptions contain some errors. In her description of *A. stellatus* larvae, Lara (2006:1390) noted that, by 5mm, most of the body is heavily pigmented except for the dorsal third, but her illustrations of 5.7 and 7.8 mm specimens depict heavy pigmentation on the dorsal third—consistent with our specimens of *A. stellatus*. She also indicated (2006:1390) that "D_1 & P_1 fins darkly pigmented," but the pectoral fin is clear in her illustrations and presumably she meant the pelvic fins: as she correctly noted later in the description, the first dorsal and pelvic fins are heavily pigmented. Likewise, in the description of *A. puncticulatus*, she noted that small larvae have large melanophores on the head and anterior half of the body, but her illustration of a 5.6 mm specimen reveals large melanophores on the posterior half of the body as well. Large melanophores are present posteriorly in our specimens. She mistakenly indicated the presence of heavy pigment on the pectoral fin as in *A. stellatus*.

One image of *A. puncticulatus* was located on the internet (Victor 2008). The 9.2-mm SL larva from San Blas, Panama, agrees well in pigmentation with our *A. puncticulatus*.

Conclusions

DNA Barcoding is an effective means of identifying the larvae of marine fishes, including those historically difficult to separate based on morphology alone. This methodology proved effective in matching larvae, juveniles, and adults of Belizean *Phaeoptyx* and *Astrapogon*. Examination of color photographs of DNA voucher specimens taken prior to preservation, as well as the voucher specimens when intact enough to be of value, proved critical in identifying distinguishing features of larvae and juveniles identified through DNA: patterns of chromatophores combined with patterns of melanophores provide the easiest means of separating early life-history stages of *Phaeoptyx* and *Astrapogon*. We note that if photographing fresh color patterns is not possible when larvae are collected, chromatophores can be preserved by fixing larvae in 5% formalin and

preserving them in ethanol plus butylated hydroxytoluene [BHT]—see Smith, 1995; Baldwin and Smith, 2003. Additional larval specimens of *P. xenus, A. alutus,* and *A. stellatus* are needed to determine diagnostic features of those species. Genetic identification of larvae of Belizean species of *Apogon* and numerous other taxa is in progress.

Acknowledgements

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